Progression of knee OA was measured by change in radiographic joint space narrowing (JSN) score (defined as any increase in JSN score, including within-grade changes) between the 24- and 48-month OAI visits, change in medial-to-lateral peri-articular bone mineral density ratio at the tibial plateau (M:L paBMD ratio), and change in four MRI trabecular morphometry measures: apparent bone volume fraction (aBV/TV), apparent trabecular thickness (aTb.Th), number (aTb.N), and spacing (aTb.Sp) between baseline and follow-up visits. We used multivariate logistic or linear regression models to analyze the associations of serum 25(OH)D and PTH levels with OA progression after adjusting for age, sex, race, study site, body mass index, dietary and supplemental intake of vitamin D, and season of blood drawn when they changed the parameter estimates ≥ 10%.

**Results:** The mean levels of serum 25(OH)D and PTH at baseline were 65. 5 nmol/L and 54.5 pg/mL, respectively. Serum 25(OH) D and PTH levels were negatively correlated (Pearson r = - 0.29, p<0.0001). Between the baseline and follow-up visits, 14.1% of the study participants had an increase in JSN score. The M:L paBMD ratio increased by 0.001 (SD=0.05). Changes in log-transformed aBV/TV, aTb.Th, aTb.N and aTb.Sp were -0.02, 0.01 mm, -0.03 $mm^{-1}$, and 0.04 mm respectively (SD=0.35, 0.09, 0.29, and 0.35). Participants with low serum vitamin D levels (<37.5 nmol/L or 15 ng/ml) had more than two-fold elevated risk of having an increase in JSN score than those with high vitamin D levels (≥37.5 nmol/L; OR=2.3, 95%CI: 1.1-4.5). Serum PTH level was not associated with increases in JSN score. Neither serum 25(OH)D nor PTH was associated with changes in M:L paBMD ratio or changes in trabecular morphometry.

**Conclusion:** Low serum level of vitamin D is associated with an increased risk of progression in joint space narrowing in knees with OA. Changes in peri-articular tibial bone mineral density and trabecular morphometry were small and not related to vitamin D or PTH levels.

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## NIGHT-TIME SPLINTING OF THE DISTAL INTERPHALANGEAL JOINT REDUCES PAIN AND IMPROVES EXTENSION AT THE JOINT: RESULTS FROM THE SPLINT-OA STUDY

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**Purpose:** Distal interphalangeal (DIP) joint osteoarthritis is common but has few cost-effective, evidence-based interventions. Pain, deformity (radial or ulnar deviation of the joint) and loss of full extension (extension lag) frequently lead to both functional and cosmetic issues. Whilst thermoplastic splinting of the first carpometacarpal joint is effective in improving pain, splinting of the interphalangeal joints is not currently an accepted therapeutic option. We investigated whether splinting the DIP joint would improve pain, function and deformity.

**Methods:** A prospective, radiologist-blinded, controlled trial of custom splinting of the DIP joint was carried out. 26 subjects with symptomatic hand osteoarthritis gave written, informed consent. All had at least 2 'affected' DIP joints (pain≥2/10 on a numerical rating scale, and at least 7° radial/ulnar deviation +/-extension lag) and were on stable therapies prior to, and during the study. One intervention joint and one control joint (which remained un-splinted - there being no satisfactory placebo) were nominated. Where possible, the contralateral, same digit DIP joint was the 'perfect match' control. A custom 'gutter' splint was worn on consecutive nights (maximum 12 hours/day) for 3 months, with clinical assessment and measurement of joint deviation (un-splinted) by digital plain radiograph at baseline and after 3 months. Statistics: Differences in change in intervention and control joint

**Table 1**
Baseline demographic, dietary and behavioral factors by serum levels of 25(OH)D (nmol/L), the Osteoarthritis Initiative, 2007-2009.

| | Total (N=488) | 25(OH)D ≥ 37.5 nmol/L (N=415) | 25(OH)D <37.5 nmol/L (N=73) | P value[1] |
|---|---|---|---|---|
| Age(yr), mean ± SD | 63.9 ± 9.1 | 64.3 ± 9.1 | 61.7 ± 9.0 | 0.02 |
| Female, % | 47.1 | 47.5 | 45.2 | 0.72 |
| Non-Hispanic white, % | 73.8 | 78.1 | 49.3 | <0.0001 |
| Body mass index ($kg/m^2$), mean ± SD | 29.7 ± 4.6 | 29.3 ± 4.5 | 31.9 ± 4.7 | <0.0001 |
| PASE score, mean ± SD | 168.1 ± 82.2 | 167.7 ± 82.0 | 170.5 ± 83.4 | 0.78 |
| Alcohol ≥1drink/day, % | 14.2 | 14.5 | 12.5 | 0.65 |
| Smokers, % | 54.8 | 56.1 | 47.2 | 0.16 |
| Dietary and supplemental intake of vitamin D ($IU/day$)[2], median (Q1-Q3) | 257.9 (102.7-444.3) | 277.9 (117.3-463.2) | 110.1 (57.8-254.4) | <0.0001 |
| Users of vitamin D supplement, % | 65.4 | 69.5 | 41.7 | <0.0001 |
| Winter/spring seasons of blood draw, % | 49.8 | 48.0 | 60.3 | 0.052 |
| History of knee injury or surgery, % | 58.6 | 59.8 | 52.1 | 0.22 |

1. P values were calculated from ANOVA (age, body mass index, PASE score), Kruskal-Wallis test (dietary intake of vitamin D) and chi-square test (sex, race/ethnicity, drinking, smoking, supplemental vitamin D use, season of blood draw and history of knee injury or surgery).
2. Values of vitamin D intake were adjusted for total energy intake.

**Table 2**
Serum levels of 25(OH)D and PTH in association with OA progression in JSN score[1], the Osteoarthritis Initiative, 2007-2009.

| | All subjects (N=418) | | Subjects with preexisting OA (N=271) | | Subjects without preexisting OA (N=147) | |
|---|---|---|---|---|---|---|
| | Not progress / Progress | OR (95%CI)[1] | Not progress / Progress | OR (95%CI)[1] | Not progress / Progress | OR (95%CI)[1] |
| 25 (OH)D (nmol/L)[2] | | | | | | |
| ≥ 37.5 | 308 / 51 | 1.0 | 190 / 38 | 1.0 | 118 / 6 | 1.0 |
| < 37.5 | 44 / 15 | 2.3 (1.1-4.5) | 28 / 15 | 3.2 (1.5-6.8) | 23 / 0 | – |
| PTH (pg/mL)[2] | | | | | | |
| < 50 | 180 / 27 | 1.0 | 109 / 24 | 1.0 | 71 / 3 | 1.0 |
| ≥ 50 | 179 / 32 | 1.1 (0.6-2.0) | 109 / 29 | 1.1 (0.6-1.2) | 70 / 3 | 0.7 (0.1-4.0) |

1. OA progression in JSN score is defined any increase in JSN score between baseline and follow-up visits.
2. Logistic regression model for serum 25(OH)D and joint space narrowing was adjusted for age (continuous) and supplemental intake of vitamin D (≥400 vs. <400 IU/day); logistic regression model for serum PTH and joint space narrowing was adjusted for age (continuous), BMI (continuous), season (summer/fall vs. winter/spring) and supplemental intake of vitamin D (≥400 vs. <400 IU/day).

outcome measures were compared by Wilcoxon Signed Ranks Test (2-tailed), firstly in the whole study population, and secondly the pre-defined subgroup with contralateral 'perfect match' control.
**Results:** Of 26 subjects, 23 were women, 3 men; mean age was 63 years (51-78); mean time from diagnosis 6.5 years; mean BMI 26. At baseline, the median average pain score in the preceding 2 weeks was similar in the intervention (6/10) and control joints (5/10). 24 subjects completed the study. A further patient required a hand steroid injection and was excluded from analysis. The average pain (primary outcome measure) and worst pain scores in the intervention joint were significantly lower at 3 months compared with baseline (p=0.002, p=0.02 respectively). Change in joint deviation on X-ray (radiologist blinded to intervention digit) approached, but did not achieve significance at 3 months (p=0.076). When nominated intervention and control joints were compared in all 23 patients, differences did not reach significance. In the subgroup who had a contralateral, 'perfect match' control digit, average pain was significantly lower in the intervention joint at 3 months (p=0.035) and extension lag was significantly improved (p=0.016). However, no significant difference in functional outcome measures, such as active range of motion or pinch grip was evident. 2 serious adverse events occurred during the study period, neither of which appeared to relate to the intervention.
**Conclusions:** DIP joint OA can lead to significant hand pain, deformity and disability. Short-term DIP joint splinting is a safe, simple, inexpensive treatment modality which reduces DIP joint pain and improves ability to extend the digit. It does not, if carried out nightly, give rise to non-compliance, increased stiffness or restriction of range of motion. The lack of a suitable placebo intervention to which investigator and patient are blinded is a recognised weakness in this type of study. Whilst powered to detect a difference in average pain, it is likely that a larger study population would be required to fully understand effects of splinting on other secondary outcomes, particularly given the large fluctuations observed in control joints. Fixed splinting, for longer periods than 3 months, may have brought about a greater change in outcomes, albeit with a stiffer joint, but is less likely to be well-tolerated. Larger trials would be required to investigate this possibility.

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## GENETIC VARIANTS NEAR INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 3 (IGFBP3) ARE ASSOCIATED WITH HIP OSTEOARTHRITIS

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**Purpose:** Hip osteoarthritis (HOA) is one of the most common joint disorders and can result in pain and disability. HOA is heritable, but the particular genes contributing to the development of HOA are not well defined. To identify genetic associations with HOA, we conducted a two-stage genome-wide association study (GWAS).
**Methods:** All analyses were restricted to individuals of European ancestry. The discovery phase was performed using radiographically determined HOA cases and controls selected from the Osteoporotic Fractures in Men (MrOS) Study and the Study of Osteoporotic Fractures (SOF) (combined cases = 662). HOA cases were defined as Croft grade $\geq$ 2 or total hip replacement (THR). HOA controls were defined as Croft grade $\leq$ 1, maximum joint space narrowing (JSN) $\leq$ 1, maximum osteophytes $\leq$ 1, and no THR. SNPs were genotyped using the Illumina Omni1-Quad array and approximately 2.5 million SNPs were imputed using the HapMap reference panel. Logistic regression was performed, and MrOS and SOF results were combined using inverse variance weighted fixed effect meta-analysis. SNP associations with P-values $\leq$ 5x10-8 were examined for replication in the Rotterdam cohorts (RSI = original cohort, RSII = second recruitment cycle) and the GOAL and Nottingham case-control studies. Publicly available eQTL data from HapMap CEU lymphoblastoid cell lines were used.
**Results:** On average, MrOS participants (100% male) were older than SOF participants (100% female) at the clinic visits when HOA was assessed (MrOS: mean$\pm$SD = 77.5$\pm$5.4 years, range = 69-97; SOF: mean$\pm$SD = 70.9$\pm$5.0 years, range = 65-91). In the discovery meta-analysis, the rs788748 A allele and the rs879966 G allele were associated with decreased odds for HOA (Table 1). The two directly genotyped SNPs were 23 kb apart and were in moderate LD (HapMap CEU r2 = 0.54). Neither SNP remained nominally significant in conditional analysis, indicating dependence. Meta-analysis of the four replication cohorts indicated that rs788748 was significantly associated with HOA (OR = 0.91, P-value = 0.01, Table 1). From eQTL data, rs788748 and rs879966 were marginally associated with IGFBP3 expression (P-value = 0.07 and 0.06, respectively), but not IGFBP1 expression (P-value = 0.74 and 0.34, respectively). Knockdown of IGFBP3 in the ATDC5 chondrogenesis model system resulted in a reduction of hypertrophic markers and a reduction in type X collagen, indicating a role for IGFBP3 in chondrogenesis.
**Conclusions:** Results from our genetic association study and IGFBP3 functional studies provide evidence for a link between IGFBP3 and HOA. IGFBP3 is known to be expressed in human chondrocytes and might be an attractive candidate for further follow-up studies.

SNP association results in discovery and replication cohorts.

| SNP | Effect allele (Freq) | MrOS/SOF (discovery) | | RSI | | RSII | | GOAL | | Nottingham | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cases/ controls | OR (P) | cases/ controls | OR (P) | cases/ controls | OR (P) | cases/ controls | OR (P) | cases/ controls | OR (P) |
| rs788748 | A (0.49) | 662/ 4750 | 0.71 (2x10-8) | 462/ 3428 | 0.86 (0.03) | 149/ 1430 | 0.98 (0.88) | 1291/ 783 | 0.85 (0.01) | 1258/ 758 | 1.01 (0.83) |

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## DIO2 IS EPIGENETICALLY REGULATED BY CPG METHYLATION IN ARTICULAR CARTILAGE

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**Purpose:** Genetic studies have identified the deiodinase iodothyronine type II gene (DIO2) as osteoarthritis (OA) susceptibility gene. DIO2 is required for the conversion of intracellular inactive thyroid hormone (T4) into active thyroid hormone (T3). During development T3 is required to induce terminal maturation of growth plate chondrocytes, leading to cell hypertrophy, extracellular matrix breakdown, mineralization of cartilage and the final formation of bone. Striking similarities between maturating chondrocytes from the growth plate and articular chondrocytes in OA cartilage have been described. Earlier we have observed a higher protein expression and allelic imbalance (AI) of DIO2 with the SNP rs225014, which is considered detrimental for the integrity of articular cartilage. In the current study, we set out to investigate whether this up regulation and AI is modulated epigenetically by CpG dinucleotide methylation and/or rs225014 genotypes.
**Methods:** Macroscopically preserved and OA cartilage was sampled from 48 Caucasian end stage OA patients who underwent total joint arthroplasty of the knee (N = 21) or hip (N = 27). After bisulphite treatment of isolated DNA from cartilage, CpG methylation was measured using Epityper, Sequenom. In total 22 CpG dinucleotides were interrogated for differential methylation between preserved and OA cartilage. Conventional RT-qPCR was used to measure gene expression. To identify functional features a Generalized Linear Mixed Model (GLMM) was fitted to model DIO2 expression from CpG methylation. Fixed covariates included joint type, disease status of cartilage and rs225014 risk allele carriage. To account for inter-individual differences a random effect for donor was included in the model. For chromatin immunoprecipitation (ChIP) primary chondrocytes isolated from three OA patients were expanded for two passages.